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Evaluation of various lignocellulosic biomass and cereal grains as potential spawn materials for wild Schizophyllum *commune* cultivation

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Abstract. Rapid mycelium growth in spawn production and on growth substrate could suppress contamination, which is significant in mushroom industry. The aim of the study is to investigate the potential of lignocellulosic biomass waste as new materials alternative to common cereal grains in producing spawn for wild S. commune cultivation on rice husk, paddy straw, and rubber wood sawdust. The fastest mycelium growth among lignocellulosic biomass was found on rice husk spawn (1.27 cm/day) and 1.98 cm/day for wheat grain. The shortest duration for substrate colonization for both lignocellulosic and grain spawn is on paddy straw, followed by rice husk, and rubber wood sawdust.

1. Introduction

Mushrooms are fleshy, spore-bearing fruiting bodies of basidiomycete fungi, typically produced above ground on soil or on its food source. In general, edible mushrooms are low in fat and calories, rich in vitamins B, D, K and sometimes vitamins A and C contain more protein than any other food of plant origin and are also a good source of mineral nutrients. Therefore, great attention has been paid on mushroom as 'functional food' to complement and supplement a healthy diet as well as for their significant role in human disease control [1]. Edible mushrooms cultivation activities in developing countries in small scale production are viable and eminent in producing food source to meet the protein requirement. Under National Agro-Food Policy (DAN), (2011-2020), mushrooms have been identified as one of the high-value commodities in Malaysia. However, the current production of mushroom is only 24 tons per day and therefore, cannot fulfils the high demand of about 50 tons per day from the local consumers [2]. In order to meet the demand, Malaysia has imported approximately 2.71 million and 3.11 million tons of fresh and dry mushroom respectively in 2012[3]. Nevertheless, there are still traditional collecting wild mushrooms activities for food and medicine among local communities as claimed by Lee SS et. al. (2009) [4]. According to them, a total of 45 species of macro fungi have been reported by Bateq, Che Wong, Jakun, Semai and Temuan communities, in which 31 species were consumed as food and 14 species were utilised as medicine including Schizophyllum commune. In East Kalimantan, Indonesia, this mushroom has become an alternative natural food source for the local

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peoples and normally can be collected from the surface of decaying palm oil empty fruit bunch wastes [5].

S. commune or locally called as "cendawan kukur" is becoming more and more popular among cultivars in Malaysia due its nutritional values, dominant characteristics, high demand, and promising high prices compared to another common cultivated mushroom such as *Pleuroteus* spp. Nevertheless, the local production of this mushroom types is still considered low. Difficulty in obtaining the commercial *S. commune* spawn is one of the recalcitrant that has slowed down the production activities of *S. commune*. Most of the local cultivars depend on the spawn supply from the neighbouring country. Therefore, cheap and readily available material such as lignocellulosic biomass should be explored in as new material in *S. commune* spawn production. Hence, the objective of the study is to analyse the mycelium growth rate of wild *S. commune* on different types of low-cost lignocellulosic biomass such as rice husk, spent empty fruit bunches, paddy straw, and rubber wood sawdust as potential materials to replace grains for *S. commune* on locally available biomass wastes such as rubber wood sawdust, rice husk, and paddy straw using the spawn from both lignocellulosic and grain spawns. To the best of our knowledge, there was no such work being performed in evaluating lignocellulosic biomass waste as material for wild *S. commune* spawn.

2. Methodology

2.1 Mushroom strain

The fresh fruiting body of *Schizophyllum commune* was collected from dead rubber trees in a rubber estate at Kampung Musa, Kuala Nerang, Kedah (6°13'43.1"N 100°40'46.6"E). The pure culture of the mushroom was maintained on Malt Extract Agar (MEA) at 30°C for further study.

2.2 Evaluation of lignocellulosic biomass and grains as potential material for spawn production

Several different grains such as maize (small and big size), sorghum, paddy, and wheat were soaked for 24 hours at room temperature and after that the water was completely drained from the samples. Then the samples were added with rice bran and CaCO₃ according to ratio 100:2:0.2. For lignocellulosic samples such as rubber wood sawdust, rice husk (grinded and original size), paddy straw, and grinded spent empty fruit bunches, these samples were dried in oven at 60°C for 48 hours prior to mixing process with rice bran and CaCO₃ according to the aforementioned ratio. The moisture contents in all samples were adjusted to $33 \pm 2\%$ using a moisture analyser (Brand Sartorius, Model MA35). After that the samples were filled into 50 ml centrifuge tubes and autoclave at 121°C for 20 minutes. After cooled down to room temperature, a 1.0 cm disc from 10 days old of wild *S. commune* grown on MEA was inserted into each centrifuge tubes and incubated at 30°C. The period for spawn run was recorded and compared for each sample. Vertical growth rate was measured as described by Rezaeian S & Pourianfar HR, (2017) [6].

2.3 Evaluation of different spawn run period of wild S. commune grown on rubber wood sawdust, rice husk, and paddy straw as artificial logs.

Each of the mother spawn types was inoculated into five different polypropylene bags (6.5' x 9.5' inches) containing half full of rubber wood sawdust, rice husk, and chopped rice straw (2.0 cm length) that were first mixed with rice bran and CaCO₃ according to the ratio 100:50:1, autoclaved at 121°C for 20 minutes, and cooled down to room temperature before spawn inoculation. Each type of growth substrates was filled until full neck with different type of spawn and were five times replicated. The inoculated artificial logs were incubated in a complete darkness at room temperature in a completely randomized design. The spawn run period (the number of days taken from inoculation until full colonization by the mycelium) for all mushroom bags were recorded and compared. The weight of the rubber wood sawdust, rice husk and paddy straw that gave the same height were 550 ± 5 g, 300 ± 5 g, and 350 ± 5 g respectively.

3. Results and Discussion

3.1 Mycelium growth rate of wild S. commune mycelium on cereal grains and lignocellulosic biomass as spawn materials.

Fig. 1 shows the mycelium growth rate of wild S. commune on cereal grains and lignocellulosic biomass for a linear growth distance of 8.6 cm, which was the length of the 50 ml centrifuge tube. For cereal grains, the fastest growth rate was observed on wheat (1.98 cm/day) followed by sorghum, paddy, maize (small), and maize (big) which were 1.89, 1.61, 1.17, and 0.92 cm/ day respectively. As for lignocellulosic materials, rice husk (1.27 cm/day) showed the highest growth rate followed by grinded rice husk (0.85 cm/day), rubberwood sawdust (0.72 cm/day), paddy straw (0.65 cm/day), and grinded empty fruit bunches (0.62 cm/day). The slower mycelium growth rate of lignocellulosic spawn compared to grains spawns may due to the facts that it was composed of lignin, cellulose, cellulose and minerals that provides a barrier for the secreted enzymes from the mycelium to digest the long chain carbohydrates into smaller compounds for its metabolism process. On the other hand, the more readily available carbohydrates in the grains provides sufficient nutrient for the mycelium growth and extension. Zhang RY et al. (2014) stated that solid spawn can be made from various grains such as rye, millet, and sorghum with an addition of gypsum or lignocellulosic biomass such as sawdust and cottonseed hulls supplemented with wheat bran and lime [7]. Dundar A et al. (2009) suggested that the variation in mycelium growth rate could, in turn due to the variations in nutritious content/ diversity and carbon to nitrogen ratio (C:N) ratio of the substrates used [8]. Higher moistureholding capacity, more exposed nutritious part, and total surface area of the cracked maize (small size) compared to the maize with bigger size might attributed to the faster growth rate of wild S. commune. The similar observations been obtained by Dulay RMR et. al., (2017) for wild hairy sawgill Lentinus strigosus spawn production using cracked corn [9].



Figure 1. Mycelium growth rate of wild *S. commune* on different types of lignocellulosic biomass and cereal grains for spawn production

3.2 Spawn run period of wild S. commune grown on rubber wood sawdust, rice husk, and paddy straw as artificial logs.

Table 1 shows the duration for full colonization of wild *S. commune* mycelial on rubber wood sawdust, rice husk, and paddy straw using different type of lignocellulosic and grain spawns. The mycelial growth extension on the different growth substrates resulting from the different types of

spawns are shown in Figure 2. The study has demonstrated that the spawn run period on rice husk and paddy straw was both comparable and faster compared to rubber wood sawdust for all types of spawns. This could be attributed to the high lignocellulosic contents of rubberwood sawdust in comparison to the other biomass types. Aditiya HB et. al., (2016) reported that the average lignin, cellulose, and hemicellulose amount of 22.2%, 31.8% and 26.8% for rice husk; and 17.2%, 37.5% and 30.35% for rice straw respectively [10]. On the other hand, Hassan SAM et. al. (2018) found that the amount of the aforementioned components was 31.52%, 33.86%, and 29.30% correspondingly [11]. The longest duration for a full colonization of wild S. commune on rubber wood sawdust was from rubber wood sawdust spawn itself (24-25 days). Applying the similar spawn types also resulted in rapid mycelium colonization on rice husk (4-15 days) and paddy straw (13-14 days) substrates. Among the grain's spawns, wheat spawn gave slightly shorter time for full colonization on rice husk substrate that was 16-17 days. Sorghum, wheat, and maize (small size) spawns resulted in 16-17 days to reach the bottom of the artificial paddy straw logs. For rubber wood sawdust growth medium, the spawn running time were almost identical for all grain's spawns (26-28 days). Higher lignin content and more compact structure of the rubber wood sawdust substrate than rice husk and paddy straw contributing to the slow mycelium growth and progress towards the bottom of the bag logs. Previous studies proved that both paddy residues have been utilized as substrates to grow many types of mushroom worldwide with high yield such as *Pleurotus ostreatus* and *Volvariella volvacea* due to its low lignin content and easily obtained locally [12], [13]. Mycelial growth rate and biomass production are related to nutrient sources and suitable C:N ratio [14].

Type of spawn	Rubber wood sawdust (a)	Rice husk (b)	Paddy straw (c)
Rice husk	27-28	15-16	13-14
Grinded rice husk	27-28	15-16	13-14
Paddy straw	27-28	15-16	14-15
Grinded empty fruit	27-28	16-17	15-16
bunches			
Rubberwood sawdust	24-25	14-15	13-14
Sorghum	26-27	17-18	16-17
Wheat	26-27	16-17	16-17
Paddy	26-27	20-21	17-18
Maize (small size)	27-28	17-18	16-17
Maize (big size)	26-27	20-21	17-18

Table. 1. Duration taken (in days) for full colonization of wild *S. commune* from different lignocellulosic and grains spawn grown on rubberwood sawdust, rice husk, and paddy straw.

Several previous studies have been conducted to cultivate *S. commune* mushroom using various lignocellulosic biomass. A study conducted by S. Ediriveera et al., (2015) proved that the mushroom fruiting bodies can be produced by using banana leaves, coconut leaves, paddy straw, and coir dust. The fastest duration of inoculation time and highest mushroom yield was obtained from coconut leaves that were 20-25 days and (9.589 \pm 0.66 g) respectively [15]. P.N. Dasanayaka and S. C. Wijeyaratne (2017) showed that the sawdust of seven different woods with supplements of rice bran, CaCO₃, and MgSO₄ can be used as growth substrate for *S. commune* fruiting bodies production [16].



4. Conclusions

From the research, we can conclude that the mycelium growth rate of wild *S.commune* on grains was more faster compared to lignocellulosic biomass. The total colonization time of lignocellulosic spawn on the three different substrates were considerably faster than grain spawn. High moisture-holding capacity, nutrient availability, and total surface area of grains attributed to the rapid mycelium growth of wild *S. commune*. Duration of spawn running on paddy straw and rice husk substrates were shorter than rubberwood sawdust due to its much lower lignin content. Shorter lag time on the new substrate and faster initial inoculation might attributed to the aforementioned observations.

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